IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	FODSTAD ET AL.	Examiner:	M. DAVIS
Serial No.:	09/331,376	Group Art Unit:	1642
Filed:	JUNE 18, 1999	Docket No.:	7885.65USWO
Title:	METHOD FOR CHARACTERIZATION OF ABNORMAL CELLS		

DECLARATION OF ØYSTEIN FODSTAD

I, Øystein Fodstad, M.D., Ph.D. am an inventor of the above-referenced application. I am head of the Department of Tumor Biology and Director, Institute for Cancer Research Council, at The Norwegian Radium Hospital, University of Oslo and have extensive experience in the biotechnical arts, including antibody production and separation. A copy of my Curriculum Vitae and a list of my recent publications is enclosed. I have read and understood the Official Actions dated July 31, 2001 and April 9, 2002 issued by Examiner Davis.

Regarding the possibility of using flow cytometry with the claimed method, we present the following experimental evidence to show the inoperability of such a modification.

Experiment 1

FEMX-1 human melanoma cells added to human blood mononuclear cells (mnc) in different ratios (10, 100, 10^3, 10^4, 10^5 melanoma cells added to 10^6 normal mononuclear cells), incubated with fluorescent yellow latex beads (2.0μm) coated with 9.2.27 anti-melanoma antibody. The cell suspensions were then used for examining and enumerating melanoma cells with bound fluorescent particles. Whereas all added cells were positive with our method with flow cytometry it was impossible to detect fewer than 10^6 melanoma cells. Actually, no reliable results were obtained by flow cytometry as the signals were spread out in a way that makes it impossible to identify the type of cells, and the number of beads on the cells.

Results:	Mixture of cells	Number of 9.2.27 positive cells Invention	Flow Cytometry
	FEMX-1 (pos. control)	100% (>5 beads/cell)	-
	Mononuclear cells (neg. control)	-	-
	10 FEMX-1/10^6 mnc	17 (170%)	no reliable results*
	100 FEMX-1/10^6 mnc	116 (116%)	
	10^3 FEMX-1/10^6 mnc		
	10^4 FEMX-1/10^6 mnc		
	10^5 FEMX-1/10^6 mnc		

* Flow cytometry gave almost no difference between 10 or 10^5 cells mixed into the mnc.

Experiment 2

Experiment 1 was repeated with Raji lymphoma cells. OS25 osteosarcoma cells were used as a negative control. 2.0 μu yellow latex beads coated with CD23 antibody were added to the cell mixtures.

Results:	Mixture of cells	Number of CD23 positive cells Invention	Flow Cytometry
	Raji (pos. control)	100% (>5 beads/cell)	-
	OS25-7 (neg. control)	-/+(some cells 1-2 beads)	-
	10 Raji/10^6 mnc	13 (130%)	no reliable results*
	100 Raji /10^6 mnc	87 (87%)	
	10^3 Raji /10^6 mnc	too many to count	
	10^4 Raji /10^6 mnc		
	10^5 Raji /10^6 mnc		

* Flow cytometry gives a positive signal for all cells with 1 or more beads bound to the surface.

The above experiments show that flow cytometry is ineffective in analyzing microsphere-labeled cells according to the instant invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such false statements may jeopardize the validity of the application or any patent issued thereon.

Date: June 14, 2002

Øystein Fodstad